Heat Treatment of Soy Flour and Its Effects on β-glucosidase Activity

Thesis

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Table of Contents

1-Introduction

1.1 Isoflavones and soy

Isoflavones, a subclass of the flavonoids, are relatively rare in the natural world. These phytochemicals can be found in certain plants, such as juniper and some mosses, but the most relevant source for human consumption of isoflavones is the soybean (Dewick 1988). Additionally, the concentration of isoflavones in soy is relatively high; up to120 μg/g and has been observed in raw, green soybeans. Comparatively, raw black beans have been shown to contain a maximum isoflavone level of 1.40 μg/g (Bhagwat and others 2008). The functions of isoflavones in plants are thought to be twofold. Studies have demonstrated that isoflavones assist with nitrogen fixation in root nodules. Additionally, these phytochemicals seem to function as a component of pathogen defense mechanisms in plants (Aoki and others 2000).

1.2-Potential Health Benefits of Soy and Isoflavones

Epidemiological studies have found a correlation between the economical development of a country and the prostate cancer mortality rates (Messina 2003). Lesser-developed countries, such as China, report less deaths associated with prostate cancer compared to developed countries, such as the United States. This difference has been partially attributed to the variance between diets of the two countries, with a focus on the amount of soybean in Asian diets (Messina 2003). Previous investigations on the correlation between the consumption of soymilk and prostate cancer risk have similarly concluded that the inclusion of soy in a human diet can lower the risk of the disease (Jacobsen and others 1998; Xu and others 1995). Furthermore, isoflavones from soy products have proven effective in animal models

with observed concentration effects (Zhou and others 1999). Based on studies such as these, it is evident that foods, such as soy, that are rich in isoflavones can positively affect health.

1.3-Isoflavone Conversion and β-glucosidase

Physiologically, isoflavones are most readily absorbed when in the aglycone form (Xu and others 1995). However, soy isoflavones exist predominantly in the less readily absorbed malonyl glycoside (MG) form, which is problematic for the development of foods meant to contain high amounts of bioavailable isoflavones (Wang and Murphy 1994). The conversion of isoflavones in the MG form to the aglycone can be encouraged via processes common to food processing, such as dry and moist heat (Coward 1998). With these processes, isoflavones can be converted to the β-glucoside form (Riedl and others 2005). The final conversion to the aglycone form must be facilitated by enzymatic cleavage of a glucose molecule from the flavonoid backbone of glycosylated isoflavones by β-glucosidase (Xu and others 1995). This enzyme is intrinsic to soy, and has demonstrated specificity to the isoflavones in the β-glucoside form (Hsieh and Graham 2001; McCue and Shetty 2003).

1.4-Inclusion of Soy in Food Systems and β-glucosidase activity optimization

A sparse amount of research exists on how to improve the delivery of the aglycone form of isoflavones to the body in the form of a food; more specifically, soy flour. When soy flour is manufactured from soybeans, β-glucosidase is activated and begins converting β-glucosides to aglycones (Pandjaitan and others 2000). Food scientists at Ohio State have taken advantage of this property of soy flour by including it as an ingredient in a model soy bread system. Riedl and

others (2005) have studied β-glucosidase activity at different proofing temperatures in the bread system with the conclusion that 48 ^{o}C represents the optimal temperature for this enzyme activity, as measured by isoflavone conversion. It is worthwhile to note that the authors did not observe complete conversion of isoflavones, which was attributed to early deactivation of β-glucosidase. This early deactivation was thought to be heat driven; however, this inactivation was seen in a dough system during proofing, and not in the raw soy ingredients.

Provided that dry and moist heat treatments can promote conversion of isoflavones to the β-glucoside form, research is needed to investigate the effects that this heat treatment elicits on isoflavone conversion and β-glucosidase activity in the soy flour used in the aforementioned model bread system. If the isoflavone conversion follows the model set forth by Riedl and others (2005) and enzyme activity is not negatively impacted, the heat treatment of soy flour, prior to its inclusion in soy bread, may prove to be a positive effector of aglycone content in the finished bread product. Therefore, the objective of the current research was to investigate the effects of the heat treatment, roasting and steaming, of soy flour with respect to β-glucosidase activity. It is expected that this heat treatment will not change enzyme activity in the soy flour.

2-Materials and Methods

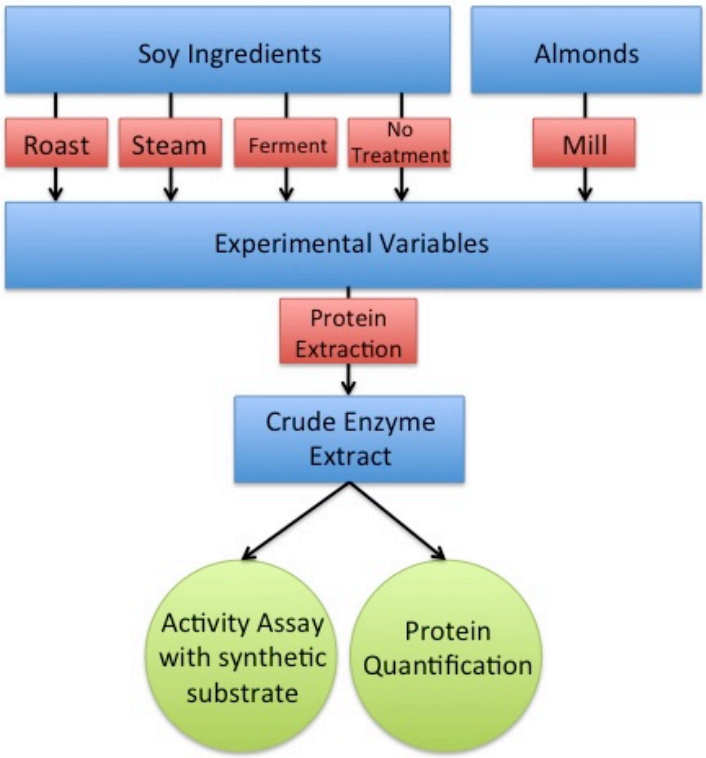

Figure 2.1. Experimental design from thermal processing through enzyme activity assays

2.1 Preparation and heat treatment of soy ingredients

The soy ingredients tested in this exercise were soy flour (Baker's soy, ADM Protein Specialties Division; Decatur, IL) and soymilk powder (Devansoy; Carroll, IA) in a 3:1 ratio. The ingredients were steamed by depositing the soy ingredient mixture on Whatman #1 filters suspended by a metal sieve above a steam bath. The steam bath was maintained for two hours to keep the soy mix temperature at 95 ± 2 ^{o}C for 120 min. Roasting of the soy ingredients was accomplished by spreading the powder onto a cookie sheet at 4 mm thickness. The ingredients were then roasted in an oven for 60 min at 175 ± 2 ^{o}C and stirred every 15 min.

Additional variables were also prepared. Soy ingredients were fermented by creating a mixture with 1:5 (w/w) of soy mix to water and storing at room temperature for three days. Raw almonds were also ground using a hand-crank mill (Porkert; Czech Republic). The almonds were passed through a #20 sieve (US standard) after grinding and prior to addition into the soy dough.

2.2 Protein (Enzyme) Extraction

Protein extracts were obtained from all samples by mixing with sodium phosphate buffer (pH 6) in a 1.0 g to 3.0 mL ratio. The resultant solutions were homogenized with a Polytron homogenizer (Kinemetica AG; Littau, Switzerland) and centrifuged at 10,000 x g for 20 min at 4 ^{o}C with a Sorvall RC5C Plus with SM-24 Rotor (Thermo Scienific; Waltham, Massachusetts). This process was completed a total of three times with the supernatant pooled over an ice bath between runs. Once the extraction was completed, the pooled extract was vacuum filtered with Whatman #4 filter paper. Extraction was also completed with the finished bread product made with each variable.

2.3 Protein Quantification of Extracts

Two separate methods of protein quantification were utilized, the Bradford protein assay and the bicinchoninic acid assay (BCA). The methods followed for these assays are available from the manufacturer, Pierce/Thermo Scientific (Waltham, Massachusetts).

2.4 β-glucosidase Activity Assay with Synthetic Substrate

A reaction mixture of 0.15 mL of 9 mM *p*-nitrophenol-β-D-glucopyranoside and 1.2 mL of sodium acetate buffer (pH 5.0) was allowed to equilibrate for 5 min at 35 ^{o}C. Following equilibration, 0.15 mL enzyme extract was added, and the reaction progress was recorded using kinetic software on a Shimadzu UV/Vis spectrophotometer set at 400 nm. The positive control utilized commercially available β-glucosidase from sweet almonds (MP Biomedicals). A blank flask was also included as a negative control in all runs. The reaction was allowed to proceed for 10 min, at which time it was stopped using 1.5 mL of 100 mM sodium carbonate solution (pH 10.0). The absorbance values obtained were compared to a seven point standard curve (10 μM to 1000 μM) generated by solubilizing *p*-nitrophenol (Fisher Scientific; Waltham, Massachusetts) into sodium acetate buffer (200 mM at pH 5.0). This assay was also performed on the finished bread product extracts. All protein assays were performed in triplicate with results reported as the mean ± standard deviation.

3-Results and Discussion

3.1 Protein Quantification

The results of the protein quantification of the soy ingredients can be seen in Table 3.1. Colorimetric assays were used in lieu of the more accurate Kjeldahl method because the main concern of this exercise was the elucidation of trends in protein content of the different variables. The need for this type of data did not justify the cost of the Kjeldahl method. From this data, it is obvious that the amount of quantifiable protein was drastically decreased from in the heat-treated variables as compared to the raw soy ingredients. The least amount of quantifiable protein was isolated from the steamed soy ingredients with a result of 5.71 and

4.54 mg bovine serum albumin/mL sample for the BCA and Bradford tests, respectively. The highest amount of protein was obtained from the raw soy ingredients at 31.5 and 38.6 mg bovine serum albumin/mL sample for the BCA and Bradford tests, respectively. The differences in protein content as detected by the two methods were attributed to the differing chemical properties of each test. The Bradford method utilizes a dye that binds to protein molecules, whereas the BCA method depends on the reduction of cupric ions by proteins for quantification (Nielson, 2003). Due to these functional differences, each test has a different detection limit.

Table 3.1. Protein quantification[a] of crude soy ingredient extracts by BCA[b] and Bradford methods.

Assay	Roast	Steam	Fermented	Raw	Almond
BCA	9.43 ± 1.29	5.72 ± 0.330	14.0 ± 3.54	31.5 ± 7.69	21.6 ± 5.16
Bradford	10.1 ± 1.88	4.54 ± 0.792	7.78 ± 1.04	38.6 ± 5.28	24.8 ± 3.92

[a]Units: mg bovine serum albumin/ mL sample
[b] Bicinchoninic Acid assay

3.2 β -Glucosidase activity

The β-glucosidase activity of the crude protein extracts from the soy ingredients and almonds was determined by a fixed end point. As expected, the almond extract and β-glucosidase enzyme standard (positive control) exhibited the highest activities, Table 3.2. The lowest activity was seen in the fermented soy ingredients with a value of 1.79 mmol p-nitrophenol/(min*g material). Surprisingly, the heat treated soy ingredients exhibited relatively high β-glucosidase activities as compared to the non-heat treated variables. This trend was also observed over time, as can be seen in Figure 3.1. From these results it was speculated that the soy β-glucosidase was thermally activated by the different heat treatments. These data also show that soy β-glucosidase was relatively heat-stable in the soy mixture, as the enzyme

activity did not degrade in the heat-treated variables. As a result, finished bread products were tested for enzyme activity. In the soy bread system, the enzyme was observed to be deactivated following baking, which demonstrated that the heat stability of soy β-glucosidase may be dependent on the type of food matrix in which it exists.

Table 3.2. β-glucosidase activity[a] of crude soy ingredient extracts determined by a fixed endpoint[b].

Variable	Mean Activity
Roasted	17.9 ± 1.94
Steamed	32.0 ± 1.7
Fermented	1.79 ± 3.02
Raw	9.06 ± 1.44
Almond	337 ± 5.01
Standard β-Glucosidase	347 ± 11.2

[a] Units: mmol p-nitrophenol/(min*g material)
[b] fixed endpoint induced by 100 mM sodium carbonate solution; read at 400 nm

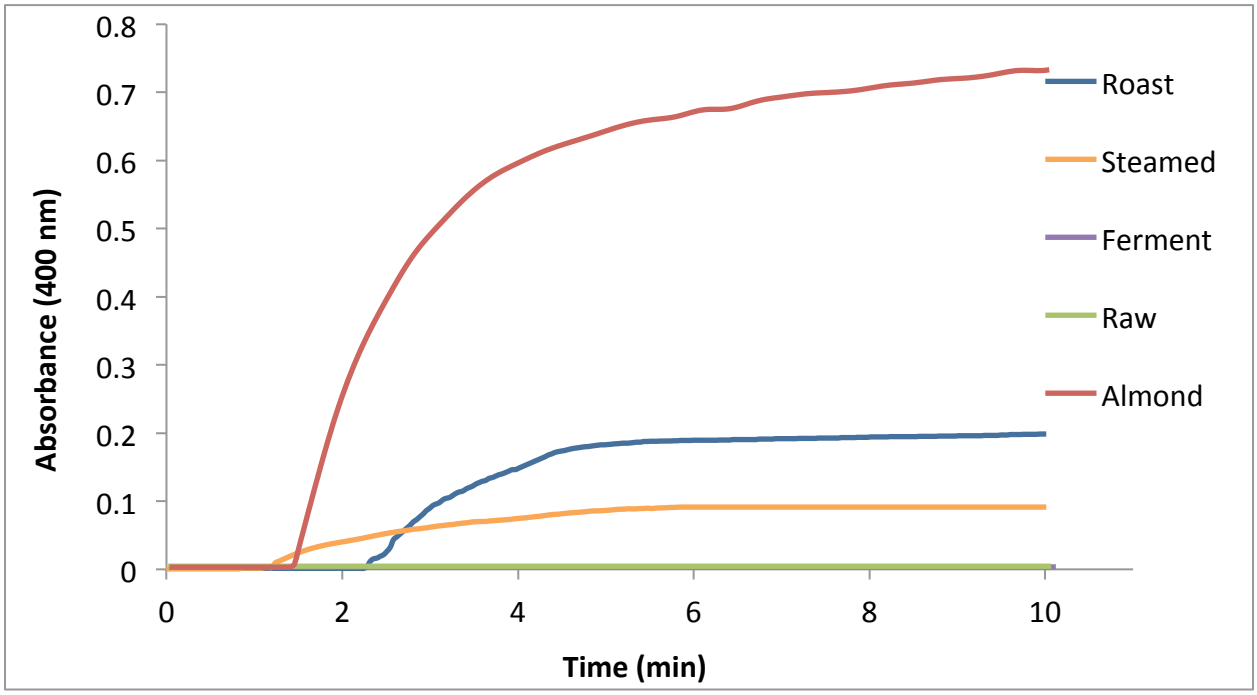

Figure 3.1. β-Glucosidase activity curves obtained from crude protein extract of soy ingredients and almonds.

4-Conclusions

From the results, it can be concluded that because of the increase in β-glucosidase activity in roasted and steamed soy ingredients, thermal processing, such as roasting and steaming, may be a means of increasing β-glucosidase activity in soy ingredients. This increase in activity occurred despite the observed decreases in protein content of the heat-treated variables. The mechanism of this heat-activation is worthy of further study. Furthermore, since β-glucosidase activity was not observed in the finished bread product, it is evident that the unique environment of the soy bread allowed for inactivation of the enzyme during baking. Therefore, the heat treatment of soy ingredients, prior to their inclusion into soy bread dough, can increase the intrinsic β-glucosidase activity of the soy to theoretically result in an increased amount of bioactive isoflavones in the finished product.

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